REMARKS

Applicants have reviewed the Final Office Action of November 1, 2005. Claims 1, 10 and 13 have been amended, and new claim 36 has been added. Claims 1-5, 7-13, 16-26, and 28-36 remain pending. Applicants request reconsideration of the application.

This application now contains independent claims (1, 3, 8, 10, 25, 28, 29, 31 and 36). Applicants will generally refer to these claims when traversing rejections.

A. Alcohol oxidase, glucose oxidase, and catalase are not described in the references to be relevant membrane enzymes.

The Examiner took the position that (i) various patents show compositions of glucose or alcohol oxidase together with azide, (ii) these are the enzymes present in the membrane fragments; and, (iii) the enzymes were still functional in the presence of the azide.

The Examiner cited US PG-Pub 2002/0045245, paragraph [0029], and Adler as defining the membrane fragments to comprise glucose oxidase and alcohol oxidase enzymes. However, paragraph [0029] does not define the membrane fragments as comprising these two enzymes. Rather, the paragraph states that these enzymes are also known biocatalytic oxygen reducing agents. Adler never states that his membrane fragments comprise these two enzymes; he never names any enzymes at all in his specification. The Examiner also incorrectly states, near the bottom of page 5 of the Office Action, that Adler teaches the use of bacterial or eukaryotic membranes containing these two enzymes. Adler teaches the use of bacterial membranes, not eukaryotic membranes; see the abstract. Bacteria are prokaryotes, not eukaryotes. Also, Adler never uses the term "eukaryotic" in his specification.

The cited patents also appear to teach that alcohol oxidase and glucose oxidase are not naturally present in bacteria. US Patent 5,081,015 (Hayashi) teaches alcohol oxidase is produced by yeast; see col. 3, lines 39-42, and col. 4, lines 25-32. So does US Patent 4,414,334; see col. 5, lines 38-60. US Patent 4,485,016 (Hopkins) teaches that glucose oxidase comes from *Aspergillus*, which is another yeast; see col. 2, lines 67-68. It is known that yeast are eukaryotes, whereas *E. coli* is a prokaryote.

The Examiner cited Hitzman (US 4,414,334) as teaching the combination of 0.02 wt% sodium azide, alcohol oxidase, and catalase, wherein enzyme activity could still be maintained. Applicants note that Hitzman obtained his enzymes from yeast; see col. 5, lines 38-60. Again, this reference does not apply to the instant claims.

Furthermore, separate and apart from the Examiner's position concerning glucose or alcohol oxidase, it is important to note that there is no relationship between these enzymes (oxidases) and respiratory enzymes. What happens to these enzymes (oxidases) in the present of azide is not predictive of what is expected to happen to the respiratory enzymes in the presence of azide. The Examiner has provided no evidence to establish this supposedly productive relationship.

Accordingly, whether the oxidases are present or not in membrane fragments is irrelevant. What is relevant is that the behavior of these enzymes in the presence of azide does not predict the affect of azide on the respiratory enzymes. It is the respiratory enzyme on the membrane fragments that generate the anaerobic environment in Applicants' invention.

B. Compositions of azide and oxygen scavenging membrane fragments are contrary to accepted wisdom in the art.

The Examiner also took the position that accepted wisdom in the art taught that azide can be added to oxygen scavenging membrane fragments and allow enzymatic activity.

Tarakhoskii was cited as teaching that the dynamic rearrangement of *E. coli* membranes during plasmolysis proceeded and was not inhibited by azide. The Examiner alleged the membranes were still functional in the presence of azide. Applicants submit the English abstract simply does not address the instant issue. The Examiner implies a relationship between the membrane ultrastructure, the lack of membrane ultrastructure change in the presence of azide, and membrane respiratory activity. This relationship is not present in the abstract. The abstract simply states that plasmolysis continues in the presence of azide. Azide can inhibit respiratory enzymes and block their function without affecting the membrane ultrastructure. The abstract does not discuss respiratory enzymes

and does not state the amount of azide used. It also does not address membrane fragments.

Milgrom was cited as teaching that anaerobic bacteria ATPase activity in *Lactobacillus* membrane fragments is only partially inhibited by 15 μM azide and thus viability is maintained. First, Milgrom discusses an anaerobic bacterium, not a facultative bacterium like *E. coli*. Second, ATPase does not appear to be an oxygen-reducing agent. ATPase catalyzes the reaction which hydrolyzes ATP into ADP plus a free phosphate ion; however, this reaction involves hydrogen, not oxygen. There is no reason to expect that the effect of azide on ATPase is predictive of the effect of azide on respiratory enzymes. Thus, Milgrom does not appear to apply to the instant claims.

Merad was cited as showing that the addition of 0.1% sodium azide to anaerobic bacteria culture media did not kill the membranes of the anaerobes, but provided growth conditions that permitted the growth of strict anaerobes over that of facultative anaerobes. Again, anaerobes will not be affected by azide because they do not contain respiratory enzymes. Merad added 0.1% sodium azide expecting to inhibit the growth of intact facultative microbes as taught by accepted wisdom in the art. However, Merad did not use separate membrane fragments to create his anaerobic environment; he used Anaerocoult P packets. Thus, he cannot teach whether accepted wisdom would expect membrane fragments to have respiratory activity.

Hope was cited as teaching that an anaerobe is viable in the presence of cyanide under anaerobic conditions and that its membranes are not killed. Anaerobic bacteria are expected to be viable in the presence of cyanide because they do not contain respiratory enzymes. Again, *E. coli* is a facultative microbe, not an anaerobe, so Hope does not apply. Hope also does not discuss membrane fragments, nor does Hope state the amount of azide used.

Tillonen was cited as teaching that azide inhibits the enzyme catalase, which is not present in strict anaerobes, and would therefore selectively inhibit catalase-containing bacteria in their growth process. While Tillonen does teach that azide inhibits catalase, the rest of the above statement is the Examiner's own reasoning and is incomplete. Tillonen also teaches that the physiological role of catalase is to protect bacteria from two toxic byproducts of oxygen, hydrogen peroxide and superoxide. See page 1113, right column,

last paragraph. One of skill in the art, reading Tillonen, would thus expect that adding azide by itself, without an oxygen reducing agent, would be detrimental to anaerobic bacteria because it would allow the buildup of toxic byproducts which also harm anaerobes. Indeed, it is known that strict anaerobes cannot survive in the presence of oxygen because of these toxic byproducts. Thus, Tillonen teaches away from adding azide. Note, in this respect, that Tillonen was interested only in measuring the amount of acetaldehyde produced by colonic contents; he was not interested in keeping anaerobes alive. Furthermore, Tillonen does not discuss the use of membrane fragments. Therefore, Tillonen does not give a true picture of accepted wisdom in the art.

Sjogren is cited as teaching that sodium azide is only moderately inhibitory of *E. coli*. Sjogren discusses sodium azide as an inhibitor of ATPase; see page 1334, right column (prior to the Discussion section). As discussed above, ATPase is not an oxygen reducing agent. Again, there is no teaching regarding the use of membrane fragments to generate anaerobic environments in the presence of azide alone.

The Examiner stated that the addition of azide to culture medium is not completely contrary to accepted wisdom in the art. To support this statement, the Examiner has provided references to yeast enzymes (the oxidases), to enzymes that do not reduce oxygen (ATPase), and to anaerobic bacteria which are not affected by azide. The Examiner's arguments do not address the novel point of Applicant's invention, which is that even with the addition of azide, the membrane fragments from bacteria sensitive to azide continue to scavenge oxygen. This activity is contrary to the prior art. For the reasons above, Applicants submit that accepted wisdom in the art would not teach the combination of azide with oxygen scavenging membrane fragments.

C. The claims are not obvious over Merad and Adler.

Claims 1-5, 7-13, 16-26, and 2-35 were rejected under 35 U.S.C. 103(a) as unpatentable over Merad in view of Adler. Applicants traverse the rejection.

There is still no motivation to combine Merad and Adler. Merad teaches that 0.1% azide inhibits the growth of facultative microbes. One of ordinary skill in the art would therefore expect that 0.1% azide would inhibit the membrane fragments of Adler. If that occurred, an anaerobic environment would not be formed. Adler would be rendered

unsuitable for his intended purpose of creating an anaerobic environment. Merad would be rendered unsuitable for his intended purpose of separating anaerobic and facultative microbes.

There is no reasonable expectation of success because accepted wisdom in the art would teach a person skilled in the art to expect the membrane fragments containing respiratory enzymes to be inhibited by azide. Neither Merad nor Adler teach that enzymes on membrane fragments are resistant to 0.1% azide. Thus, an anaerobic environment would not be formed and there would be no reasonable expectation of recovering anaerobic microbes. Therefore, the claims are not obvious based on Merad and Adler.

The Examiner's replies to these arguments, in items 2 and 3 of the Final Office Action, are premised on the prior art teaching compositions of alcohol oxidase and/or glucose oxidase with azide which still permitted enzymatic activity. However, as discussed above, the behavior of the enzymes is not predictive of the behavior of the respiratory enzymes. Because the premise of the Examiner's replies does not hold, neither can the Examiner's conclusion of obviousness.

For these reasons, Applicants request withdrawal of the 103(a) rejection based on Merad and Adler.

In paragraphs 4 and 5 of the Office Action, the Examiner stated that Applicants asserted that growth media of Merad and Adler lacked a hydrogen donating substance. This is incorrect. Applicants made this assertion against the combinations of Merad with Copeland or Fung. Applicants agree that Adler explicitly teaches a hydrogen donor and did not mischaracterize it. Applicants merely interpreted Merad differently from the Examiner.

D. The claims are not obvious over Merad and Copeland.

The 103(a) rejection based on Merad and Copeland (US 5,830,746) was maintained. Applicants traverse the rejection.

The combination of Merad and Copeland does not render the instant claims obvious for the same reasons given above. Again, the Examiner cites Copeland solely for the use of membrane fragments. Copeland does not teach that membrane fragments are resistant to azide. Therefore, the claims are not rendered obvious.

Method claims 8, 29, and 31 all teach the use of an inhibitor in the liquid broth. Neither reference discloses this claim limitation. Claims 8 and 29-35 are non-obvious for this additional reason.

For these reasons, Applicants request withdrawal of the 103(a) rejection based on Merad and Copeland.

E. The claims are not obvious over Merad and Fung.

The 103(a) rejection based on Merad and Fung (US 5,405,773) was maintained. Applicants traverse the rejection.

The combination of Merad and Fung does not render the instant claims obvious for the same reasons given above. Again, the Examiner cites Fung solely for the use of membrane fragments. Fung does not teach that membrane fragments are resistant to azide. Therefore, the claims are not rendered obvious.

Method claims 8, 29, and 31 all teach the use of an inhibitor in the liquid broth. Neither reference discloses this claim limitation. Claims 8 and 29-35 are non-obvious for this additional reason.

Fung also teaches away from the use of an inhibitor because he is trying to encourage the growth of the facultative pathogen *L. monocytogenes*. See the abstract.

For these reasons, Applicants request withdrawal of the 103(a) rejection based on Merad and Fung.

F. Some claims have been amended.

Claims 10 and 13 have been amended so that claim 10 now recites the membrane fragments are derived from bacteria and claim 13 recites the membrane fragments are derived from a specific bacterium *E. coli*. Basis for the amendment lies in original claims 13 and 15. The prior amendment of August 5, 2005 incorrectly amended these claims. Claim 10 is distinct from claim 20 in that it recites a different amount of inhibitor. Applicants take the position that the term "terminate" is different from "limit" because it connotes a complete limiting.

New claim 36 has been added. This claim finds support in original claim 20 (which recited the mitochondrial organelles). Applicants note that this claim is distinguished from

Blondin by the recitation of a nutrient medium. A review of Blondin finds that Blondin's invention would be rendered unsuitable for its intended purpose if a nutrient medium were added to it.

Upon review of the response filed August 8, 2005, Applicants made the statement on page 10, 4th paragraph, that "neither Adler nor Merad teaches the use of a liquid medium." Applicants retract that statement. The patentability of the instant claims do not depend on that limitation.

Applicants note that each of the various limitations made as part of the claim amendments have been present in at least one of the claims throughout the prosecution of this application, as have their combinations. Applicants therefore believe that additional search and reexamination is not required in light of the references already cited by the Examiner; however, the Examiner is welcomed to do so.

CONCLUSION

In view of the above amendments and comments, Applicants submit the pending claims are in condition for allowance. Withdrawal of the rejections and issuance of a Notice of Allowance is requested.

In the event the Examiner considers personal contact advantageous to the disposition of this case, she is hereby authorized to call Richard M. Klein at telephone number 216-861-5582, Cleveland, Ohio.

Respectfully submitted,

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April 3, 2006
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